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Minimal model for signal-induced Ca^{2+} oscillations and for their frequency encoding through protein phosphorylation

(Ca^{2+} signaling/inositol 1,4,5-trisphosphate/biochemical oscillations/Ca^{2+}-activated protein kinase)

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ABSTRACT **In a variety of cells, hormonal or neurotransmitter signals elicit a train of intracellular Ca^{2+} spikes. The analysis of a minimal model based on Ca^{2+}-induced Ca^{2+} release from intracellular stores shows how sustained oscillations of cytosolic Ca^{2+} may develop as a result of a rise in inositol 1,4,5-trisphosphate ($InsP_3$) triggered by external stimulation. This rise elicits the release of a certain amount of Ca^{2+} from an $InsP_3$-sensitive intracellular store. The subsequent rise in cytosolic Ca^{2+} in turn triggers the release of Ca^{2+} from a second store insensitive to $InsP_3$. In contrast to the model proposed by Meyer and Stryer [Meyer, T. & Stryer, L. (1988) *Proc. Natl. Acad. Sci. USA* 85, 5051–5055], the present model, which contains only two variables, predicts the occurrence of periodic Ca^{2+} spikes in the absence of $InsP_3$ oscillations. Such results indicate that repetitive Ca^{2+} spikes evoked by external stimuli do not necessarily require the concomitant, periodic variation of $InsP_3$. The model is closely related to that proposed by Kuba and Takeshita [Kuba, K. & Takeshita, S. (1981) *J. Theor. Biol.* 93, 1009–1031] for Ca^{2+} oscillations in sympathetic neurones, based on Ca^{2+}-induced Ca^{2+} release. We extend their results by showing the minimal conditions in which the latter process gives rise to periodic behavior and take into account the role of the rise in $InsP_3$ caused by external stimulation. The analysis further shows how signal-induced Ca^{2+} oscillations might be effectively encoded in terms of their frequency through the phosphorylation of a cellular substrate by a protein kinase activated by cytosolic Ca^{2+}.**

Hormonal or neurotransmitter signals evoke repetitive spikes of intracellular Ca^{2+} in a variety of cells such as hepatocytes (1, 2), pituitary gonadotropes (3) and somatotropes (4), endothelial cells (5), and fibroblasts (6) as well as in eggs upon fertilization (7, 8). This oscillatory phenomenon is characterized by periods ranging from <1 s to some 30 min, depending on the cell type (9, 10). The ubiquity of signal-induced Ca^{2+} oscillations suggests that the effects of second messengers might be encoded primarily by the frequency of their periodic evolution (2–6, 9, 10). Here we present a simple theoretical model for signal-induced Ca^{2+} oscillations and show how the latter may be effectively transduced into a frequency-dependent cellular response through protein phosphorylation by a Ca^{2+}-activated kinase.

What is the origin of the repetitive Ca^{2+} spikes evoked by extracellular stimuli? Inositol 1,4,5-trisphosphate ($InsP_3$) mediates the action of many external signals in inducing the release of Ca^{2+} from intracellular stores (11). It has been suggested (2) that Ca^{2+} oscillations originate from a negative feedback exerted by this ion on phosphatidylinositol-specific phospholipase C. An alternative, theoretical model (12), which also relies on $InsP_3$ oscillations, predicts the occurrence of Ca^{2+} spikes because of cross-catalytic interactions between the two second messengers. On the other hand, Berridge and colleagues have suggested (9, 10, 13) that the oscillations in Ca^{2+} may arise solely from the cycling of this ion between the cytosol and a pool insensitive to $InsP_3$, once a certain amount of Ca^{2+} has been released into the cytosol from a second pool, after the rise in $InsP_3$ triggered by the external signal. On the basis of their experimental results, Jacob *et al.* (5) considered a variety of possible mechanisms for Ca^{2+} spiking and ruled out the cellular membrane potential as a source for the observed oscillations. Likewise, the injection of $InsP_3$ into cells has been shown to elicit oscillatory Ca^{2+} transients, suggesting that the periodic generation of $InsP_3$ can be bypassed as a source of repetitive Ca^{2+} spikes (13–16). The model presented below indicates that repetitive spikes of Ca^{2+} may arise from a steady increase in $InsP_3$ elicited by external stimulation, on the basis of the self-amplified release of Ca^{2+} from intracellular stores. That the latter process might lead to Ca^{2+} oscillations has been considered for nerve (17) and cardiac cells (18–20). Here we generalize these studies and extend them by taking the role of $InsP_3$ explicitly into account.

Minimal Model for Signal-Induced Ca^{2+} Oscillations

Following Berridge (9, 10, 13), we assume that the external signal triggers the synthesis of $InsP_3$, whose effect is merely to cause the discharge of an intracellular pool of Ca^{2+}, the level of which ion thereby rises in the cytosol (Fig. 1). To determine whether oscillations can occur solely by the cycling of Ca^{2+} between the cytosol and the $InsP_3$-insensitive pool, we make the simple hypothesis that as long as the stimulus is present, its effect is to produce a net, constant influx of Ca^{2+} from the $InsP_3$-sensitive pool. This pool is considered to remain constant as a result of rapid replenishment by influx of extracellular Ca^{2+} through an autoregulatory mechanism controlled by the Ca^{2+} content of the $InsP_3$-sensitive pool (21, 22) and by the uptake of cytosolic Ca^{2+} after a spike (Fig. 1). Such refilling of the $InsP_3$-sensitive pool may be favored by inositol 1,3,4,5-tetrakisphosphate ($InsP_4$), a metabolic product of $InsP_3$ (23). However, formation of $InsP_4$ is not necessary to establish oscillations because these have been observed in pancreatic cells after perfusion with a nonmetabolizable analogue of $InsP_3$ (16). The magnitude of the influx, $v_1\beta$, from this $InsP_3$-sensitive pool is taken as proportional to the saturation function β of the receptor for $InsP_3$ [the cooperative nature of this saturation function (24) is expressed implicitly in β]; the level of $InsP_3$ established upon stimulation increases with the magnitude of the external signal. Thus, $InsP_3$ regulates the flow of Ca^{2+} into the cytosol, which primes the $InsP_3$-insensitive pool for oscillatory cycles of Ca^{2+} release (Fig. 1).

The two variables of the model are the concentration of free Ca^{2+} in the cytosol and in the $InsP_3$-insensitive pool

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Abbreviation: $InsP_3$, inositol 1,4,5-trisphosphate.

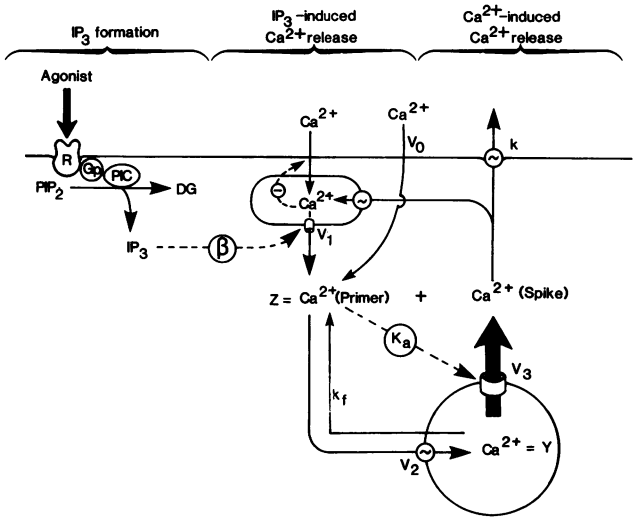

FIG. 1. Schematic representation of the mechanism generating Ca^{2+} oscillations, based on the self-amplified release of Ca^{2+} from intracellular stores. Agonists act through a transducing mechanism comprising a receptor (R), a G-protein (Gp), and phosphatidylinositol-specific phospholipase C (PIC) to hydrolyze the inositol lipid phosphatidylinositol 1,4-bisphosphate into diacylglycerol (DG) and $InsP_3$ (IP_3). $InsP_3$ modulates the release of Ca^{2+} from an $InsP_3$-sensitive store into the cytosol (v_1) and indirectly controls the influx of external Ca^{2+} into this store through the capacitative mechanism proposed by Putney (21). In the model, therefore, each level of $InsP_3$ controls a constant flow of Ca^{2+} into the cytosol, determined by $v_1\beta$. The concentration of cytosolic Ca^{2+} (Z) passes through two phases, one of low concentration, during which primer Ca^{2+} is being transferred (v_2) into the $InsP_3$-insensitive pool, interspersed with brief Ca^{2+} spikes when Ca^{2+} stored in that pool (Y) is released to the cytosol (v_3) in a process activated by cytosolic Ca^{2+}. Parameters v_0, k, and k_f relate respectively to the influx of extracellular Ca^{2+} into the cytosol, to the efflux of cytosolic Ca^{2+} from the cell, and to a passive leak of Y into Z. See text for further details.

(e.g., the endoplasmic or sarcoplasmic reticulum); these variables are denoted by Z and Y, respectively. When assuming that buffering is linear with respect to Ca^{2+} concentration, the time evolution of the system is governed by the two kinetic Eqs. 1:

$$\frac{dZ}{dt} = v_0 + v_1\beta - v_2 + v_3 + k_f Y - kZ$$
$$\frac{dY}{dt} = v_2 - v_3 - k_f Y. \qquad [1]$$

In these equations, v_0 and kZ relate, respectively, to the influx and efflux of Ca^{2+} into and out of the cell, which take place even in the absence of external stimulation; for simplicity, these terms are taken as constant and linear, respectively. The rate of ATP-driven pumping of Ca^{2+} from the cytosol into the $InsP_3$-insensitive store is denoted v_2, while v_3 represents the rate of transport from this pool into the cytosol; the term $k_f Y$ refers to a nonactivated, leaky transport of Y into Z (although not essential for the occurrence of oscillations, this process was found to stabilize the amplitude of Ca^{2+} transients at different levels of stimulation). In the above equations, all concentrations and rates are defined with respect to the total cell volume.

When the cell receives an external signal, this triggers an increase in $InsP_3$, which leads to a rise in the saturation function β and, subsequently, to an increase in cytosolic Ca^{2+}. We wish to determine the conditions in which this initial rise elicits a train of Ca^{2+} spikes. As the system of Eqs. 1 comprises only two variables, it is possible to resolve this question by resorting to phase plane analysis (25). In particular, the Bendixson criterion indicates that activation of v_3 by Z is most appropriate for inducing sustained oscillations upon external stimulation (26). This condition corresponds to an activation by cytosolic Ca^{2+} of the transport of Ca^{2+} from the intracellular store into the cytosol. Evidence in favor of such control has been obtained in skinned cardiac cells (27) and sarcoplasmic reticulum vesicles (28) and could provide the molecular basis for the observed Ca^{2+}-induced Ca^{2+} release described for muscle (29) and cardiac (30) cells as well as oocytes (31). An alternative view (9, 10, 13, 18) attributes the latter phenomenon to the progressive filling of the intracellular store, which is triggered to release its Ca^{2+} when the concentration within the store reaches a threshold for secretion. The present model predicts that at least in the absence of time delays, such a process does not suffice to induce a sustained oscillatory response.

When taking into account the cooperative nature of the pumping process and of the Ca^{2+} release from the intracellular store and the positive feedback exerted on the latter transport by cytosolic Ca^{2+}, the rates v_2 and v_3 in Eqs. 1 take the form given by Eqs. 2:

$$v_2 = V_{M2}\frac{Z^n}{K_2^n + Z^n}, \qquad v_3 = V_{M3}\frac{Y^m}{K_R^m + Y^m}\cdot\frac{Z^p}{K_A^p + Z^p}. \qquad [2]$$

In these equations, V_{M2} and V_{M3} denote respectively the maximum rates of Ca^{2+} pumping into and release from the intracellular store; these processes are described by Hill functions whose cooperativity coefficients are taken as n and m; p denotes the degree of cooperativity of the activation process; K_2, K_R, and K_A are threshold constants for pumping, release, and activation.

Eqs. 1 and 2 admit a unique steady-state solution. Linear stability analysis (25) of these equations indicates that the steady state is not always stable. It is well known from studies of physicochemical systems that sustained oscillations of the limit-cycle type develop precisely when the nonequilibrium steady state becomes unstable (32).

We consider a situation in which the system, in the absence of stimulation, is initially in a stable steady state characterized by a low cytosolic Ca^{2+} level close to 0.1 μM. Fig. 2 *Upper* shows how the system responds to an increase in β up to 30% due to a rise in $InsP_3$ triggered by the external signal. Repetitive spikes of cytosolic Ca^{2+} occur (solid line in Fig. 2 *Upper*); these oscillations are accompanied by a sawtooth variation of the Ca^{2+} content of the intracellular store (dashed line in Fig. 2 *Upper*). The waveform of the cytosolic Ca^{2+} spikes resembles that observed in some experiments; in particular, a progressive rise in Ca^{2+} often precedes the Ca^{2+} spike (5). The period of the oscillations in Fig. 2 is of the order of 1 s, as in some experimental systems (3, 4, 18). Periods of the order of 1 min or more observed in other cells (1, 2, 5, 6) are readily obtained when the kinetic parameters are divided by a factor of 10–100; such variations of Ca^{2+} pumping and transport rates in different cell types are rather common (33) and may result in part from differences in the volumes of various cells and intracellular stores.

A characteristic feature of the oscillations in liver, and perhaps also in other cells, is that the shape of each spike can vary depending on the agonist employed (1, 2). For example, norepinephrine gave narrow symmetrical transients, whereas those induced by vasopressin were much broader (1, 2, 34). Rooney *et al.* (34) have suggested that this broadening of the spike may be explained by the agonist having the additional effect of inhibiting the extrusion of Ca^{2+} (k). There already is experimental evidence that agonists can inhibit the plasma membrane Ca^{2+} pump, with vasopressin being more effective than norepinephrine (35, 36). When we reduced the Ca^{2+}

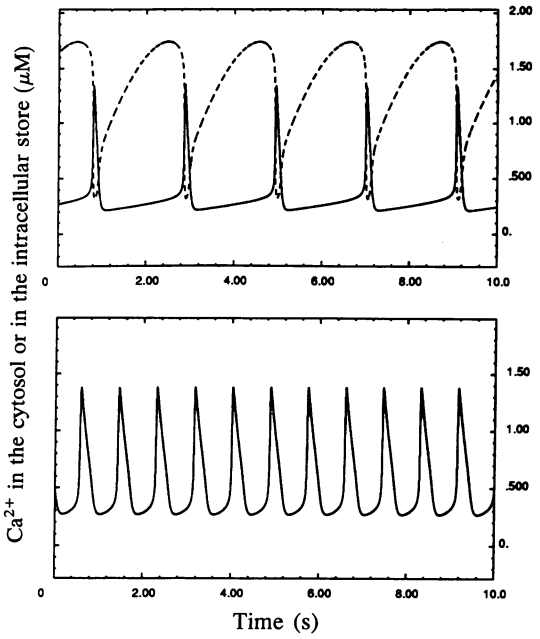

FIG. 2. (*Upper*) Oscillations in cytosolic Ca^{2+} (Z; solid line) brought about by an increase in β up to 30.1% triggered by external stimulation. The concentration of Ca^{2+} in the InsP_3-insensitive intracellular store (Y; dashed line) has a concomitant sawtooth variation; for the sake of clarity, the actual level of Y (defined with respect to the total cell volume) has been decreased by 0.35 μM. The curves are obtained by integration of Eqs. **1** and **2** for the following parameter values, which are in a physiological range (33): $v_0 = 1$ μM·s^{-1}, $k = 10$ s^{-1}, $k_f = 1$ s^{-1}, $v_1 = 7.3$ μM·s^{-1}, $V_{M2} = 65$ μM·s^{-1}, $V_{M3} = 500$ μM·s^{-1}, $K_2 = 1$ μM, $K_R = 2$ μM (defined with respect to the total cell volume), $K_A = 0.9$ μM, $m = n = 2$, and $p = 4$. Oscillations also occur, for different parameter values, with $m = n = p = 1$. (*Lower*) Oscillations of cytosolic Ca^{2+} obtained for the smaller value of the Ca^{2+} extrusion rate constant, $k = 6$ s^{-1}; other parameter values are as in *Upper*.

extrusion rate constant k from 10 to 6 s^{-1}, there was a significant enlargement of the Ca^{2+} spikes mainly because of slower decay (Fig. 2 *Lower*), which thus could account for the shape of the transients observed experimentally with some agonists (1, 2, 34). The broadening of the peaks obtained by decreasing the extrusion rate constant at a given value of β was accompanied by an increase in frequency (compare Fig. 2 *Upper* and *Lower*), which is a theoretical prediction.

How the period of Ca^{2+} oscillations varies with the extent of stimulation, as measured by β, is depicted in Fig. 3. The results agree qualitatively with experimental observations. Thus, the initiation of Ca^{2+} spikes and the rise in their frequency upon increasing stimulation are observed in a variety of cells (1–6, 9, 10, 18). The final disappearance of the spikes with the concomitant establishment of a high, constant level of cytosolic Ca^{2+} is also observed, for example in endothelial cells stimulated by a sufficiently large amount of histamine (5). The amplitude of Ca^{2+} oscillations, measured by the maximum Ca^{2+} level, is of the order of 1–1.5 μM; like the half-width of the Ca^{2+} spikes (not shown), it remains practically independent of the extent of stimulation (Fig. 3), in agreement with experimental observations (2, 34).

An important property of the present model is that repetitive Ca^{2+} spikes can occur in the absence of InsP_3 oscillations. Such a situation is supported by a number of experimental observations (13–16), including those on skinned cardiac cells (18), which lack InsP_3 production. The model further indicates how oscillations may progressively vanish (5) in the absence of external Ca^{2+}: bringing the value of v_0 down to zero eventually suppresses the oscillations; the hypothesis of a constant input $v_1\beta$ from the InsP_3-sensitive

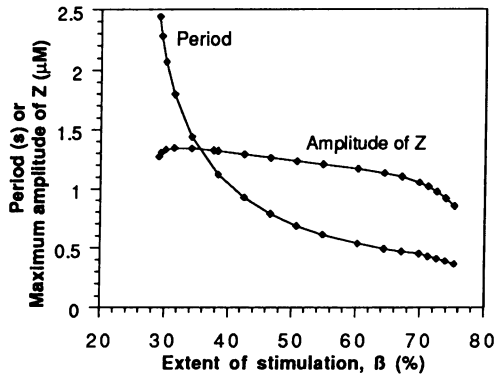

FIG. 3. Period and amplitude of cytosolic Ca^{2+} oscillations as a function of the saturation function β of the InsP_3 receptor, which rises with the external stimulation. For the values of v_0 and v_1 given in Fig. 2, oscillations occur when β ranges from 29.1% to 77.5%. A stable steady state is reached outside this range; this state corresponds to a low value of cytosolic Ca^{2+} for $\beta < 29.1\%$ and to a larger value, close to 0.7 μM, for $\beta > 77.5\%$. Similar results are obtained when plotting the amplitude and the period as a function of the influx of extracellular Ca^{2+}, v_0, for a given value of $v_1\beta$. Points denote results obtained by numerical integration of Eqs. **1** and **2** for the parameter values of Fig. 2.

Ca^{2+} store also ceases to hold in these conditions, as this store can no more be replenished. On the other hand, since the bifurcation parameter whose increase brings about the oscillations is the sum $(v_0 + v_1\beta)$, it can readily be seen that oscillations can be triggered by an increase in β due to stimulation by an external signal, or simply by an increase in v_0 originating from an increase in extracellular Ca^{2+}. The analysis suggests that for sufficiently large values of v_0 or for sufficiently low values of k, repetitive Ca^{2+} spikes may develop spontaneously (i.e., in the absence of external stimulation). This situation appears to occur, for example, in growth hormone-secreting somatotropes (4) and cardiac cells (37). The results of Fig. 3 also agree with the experimental observation (6, 38, 39) that the frequency of oscillations rises when the level of extracellular Ca^{2+} increases.

Ca^{2+} spiking can also be induced by caffeine (38). It has been argued (10) that this effect is due to a decrease in the threshold constant K_R for the release of Ca^{2+} from the intracellular store. Such a view holds with the observation that a decrease of K_R in the model can induce oscillations; further decrease in this parameter leads to an increase in frequency and a decrease in the amplitude of the spikes, as observed when increasing the stimulation by caffeine (10).

Useful information has been gained from experiments on the effect of perturbations by cytosolic pulses of Ca^{2+} or InsP_3 in fibroblasts undergoing Ca^{2+} oscillations (6). The model accounts for the observation (6) that large pulses of Ca^{2+} reset the oscillations at all phases by inducing the cell to behave as if it started at a normal Ca^{2+} peak; similar phase advances have been induced by Ca^{2+} pulses in sympathetic neurones (40). The model also predicts that instead of the phase advance obtained with larger pulses, delays can be induced after completion of a Ca^{2+} spike by subthreshold pulses of Ca^{2+}, just below those inducing significant release from the intracellular store; the transient suppression of Ca^{2+} oscillations by a critical Ca^{2+} pulse has also been achieved. These effects, which can be accounted for by phase plane analysis (G.D. and A.G., unpublished data), have not yet been observed.

Frequency Encoding Through Protein Phosphorylation

The idea is emerging that Ca^{2+} oscillations might be encoded in terms of their frequency (2–6, 9, 10, 12). The advantages

of such encoding have been discussed for other modes of intercellular communication (41–43). How could Ca^{2+} oscillations be translated into a frequency-dependent cellular response? One likely way is through protein phosphorylation. Consider a kinase activated in a Michaelian manner by cytosolic Ca^{2+} and a phosphatase, which both act on a protein substrate, W, whose fraction in the phosphorylated form is denoted W^*. The time variation of W^* associated with the oscillations of cytosolic Ca^{2+} (Z) is given by Eq. 3 (see ref. 44 and the legend to Fig. 4):

$$\frac{dW^*}{dt} = (v_P/W_T)\left[(v_K/v_P)\,\frac{1-W^*}{K_1+1-W^*} - \frac{W^*}{K_2+W^*}\right] \quad [3]$$

with

$$v_K = V_{MK}\,\frac{Z}{K_a+Z}\,.$$

Owing to the periodic variation in cytosolic Ca^{2+} (Z) and to the subsequent variation in kinase activity, the value of W^* periodically rises and decreases with Z (Fig. 4). A comparison of curves a and b in Fig. 4 shows that the mean level of W^* increases with the frequency of Ca^{2+} oscillations (i.e., with the level of stimulation, measured by β); this effect is partly due to a change in the mean value of Z, which is higher at the larger value of β considered. Equally significant, however, is the fact that when the period of oscillations is longer, the protein undergoes significant dephosphorylation from one Ca^{2+} peak to the next; much less dephosphorylation occurs between successive Ca^{2+} spikes at the higher frequency of oscillations, resulting in the maintenance of a larger fraction of protein phosphorylated.

Efficient frequency encoding through protein phosphorylation only occurs, however, in precise kinetic conditions. The analysis indicates that saturation of the converter enzymes [which favors the occurrence of "zero-order ultrasensitivity" (44)] allows for a better encoding of Ca^{2+} oscillations as the mean value of the fraction of phosphorylated protein varies over a much larger range (0.2–0.95) with the frequency of Ca^{2+} oscillations than it does when the kinase and the phosphatase are not saturated by their substrate (compare curves a and b obtained for the same oscillations in Fig. 5).

The analysis further shows that to obtain a significant variation in phosphorylated protein, cytosolic Ca^{2+} would have to oscillate in such a manner that the ratio v_K/v_P goes above a threshold (here equal to unity; see ref. 44) when Z reaches its maximum, and below it when Z decreases to its minimum. Such fine tuning clearly depends on the absolute and relative values of the maximum rates of the kinase and phosphatase, of their Michaelis constants, and on the value of the activation constant K_a, which corresponds to the Ca^{2+} concentration that yields the half-maximum kinase activity.

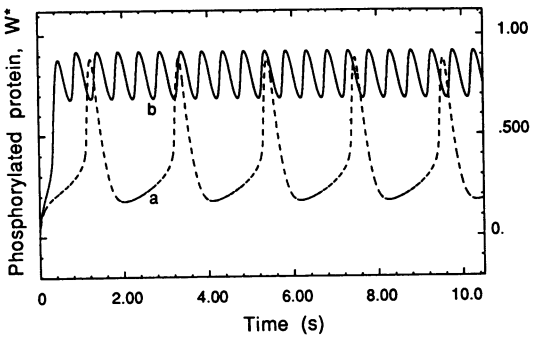

FIG. 4. Oscillations in the level of a protein phosphorylated by a kinase activated by cytosolic Ca^{2+}. Curve a is the time evolution of the fraction of phosphorylated protein in the course of the Ca^{2+} oscillations shown in Fig. 2 *Upper*; $\beta = 30.1\%$, and the mean fraction of phosphorylated protein over a period, $\langle W^* \rangle$, is equal to 0.32. Curve b is the time evolution of protein phosphorylation at a higher level of stimulation; $\beta = 64.4\%$ and $\langle W^* \rangle = 0.80$. The curves are obtained by integration of Eqs. 1–3 with $W^* = 0$ at time zero for $v_P = 5\ \mu M \cdot s^{-1}$, $V_{MK} = 40\ \mu M \cdot s^{-1}$, $K_a = 2.5\ \mu M$, $W_T = 1\ \mu M$, and $K_1 = K_2 = 0.1$; these values are in the physiological range for protein phosphorylation systems (45). For these parameter values, the phosphorylation curve for W^* possesses a sharp threshold, where $W^* = 0.5$, in $v_K/v_P = 1$ (see ref. 44). In Eq. 3, v_K and v_P denote the maximum rates of kinase and phosphatase at a given value of Z; V_{MK} is the maximum rate of the kinase at saturation by Z; K_a denotes the constant of activation of the kinase by cytosolic Ca^{2+}; $K_1 = K_{m1}/W_T$ and $K_2 = K_{m2}/W_T$, where K_{m1} and K_{m2} denote the Michaelis constants of kinase and phosphatase, while W_T is the total amount of protein substrate.

Discussion

The above analysis of a simple two-variable model shows that repetitive Ca^{2+} spikes may be triggered by external stimulation solely on the basis of the self-amplified release of Ca^{2+} from intracellular stores. For oscillations to occur, the latter process should involve the activation of the release by cytosolic Ca^{2+} rather than the filling up of intracellular stores beyond a threshold level. Oscillations are brought about by the rise in cytosolic Ca^{2+} that is itself induced by the increase in $InsP_3$ that follows stimulation. Some stimulations are too weak to produce the rise in β up to the level required for oscillations. This might explain, for example, why in fibroblasts a depolarization by gramicidin and a stimulation by vasopressin are required for inducing oscillations, regardless of the order of the two stimulations; none of the stimuli by themselves can induce repetitive spikes (6). The model suggests that only the combined stimuli raise cytosolic Ca^{2+} sufficiently, so that the value of $(v_0 + v_1\beta)$ increases above the critical threshold required for oscillatory behavior. The single Ca^{2+} spike evoked by each stimulus (6) could represent an excitable response.

Besides accounting for a large body of experimental observations, a number of testable predictions were made pertaining to the effect of the parameters on the shape and period of Ca^{2+} transients. In the model, an increase in $(v_0 + v_1\beta)$ will accelerate the oscillations by shortening the phase of cytosolic Ca^{2+} accumulation up to the threshold level at which self-amplified release begins, while an increase in k or V_{M2} will slow down the production of spikes by increasing the time interval required for triggering Ca^{2+} release from intracellular stores. Another prediction concerns the existence of critical pulses of Ca^{2+} producing phase delays similar to those

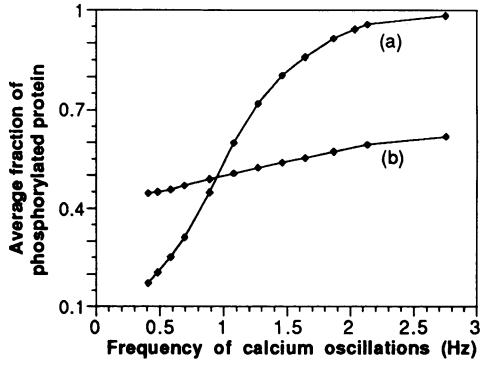

FIG. 5. Frequency encoding of Ca^{2+} oscillations through protein phosphorylation. The curves show the average value of the fraction of phosphorylated protein, $\langle W^* \rangle$, over a period in the course of the Ca^{2+} oscillations in the range given in Fig. 3. Data (points) are obtained as in Fig. 4 for the same parameter values, with $K_1 = K_2 = 0.01$ (curve a) or 10 (curve b).

observed (6) with InsP_3. Finally, although this minimal model cannot account for all variations of Ca^{2+} oscillations in response to different ligands, it indicates, however, how each agonist might possess its own "signature" by acting at the same time, in a specific manner, on several parameters such as β and k.

The present model for Ca^{2+} oscillations differs from that proposed by Meyer and Stryer (12) in that it comprises only two variables (instead of three), does not require InsP_3 oscillations for Ca^{2+} spiking or Ca^{2+} pumping into mitochondria, and incorporates the effect of extracellular Ca^{2+}. On the other hand, the above model is closely related to that proposed by Kuba and Takeshita (17) for membrane potential oscillations linked to Ca^{2+} spikes in sympathetic neurones treated with caffeine. One of the models proposed by these authors contains two variables and also relies on the Ca^{2+}-induced Ca^{2+} release mechanism to produce oscillations. Here, the effect of the external stimulation and of InsP_3 is taken explicitly into account, and the source of the instability leading to oscillations is made clear by the analysis of the minimal conditions that produce the phenomenon. While Kuba and Takeshita considered activation of the release by both cytosolic Ca^{2+} and Ca^{2+} in the intracellular store, we showed that the former control suffices for producing oscillations. In fact, the suggestion that oscillations may arise from Ca^{2+}-induced Ca^{2+} release was first made for cardiac cells in which this mechanism may operate as one of those generating pacemaker behavior (18–20).

That signal-induced Ca^{2+} spikes are observed in a large variety of cells suggests the robustness of the mechanism underlying these oscillations. The activation by cytosolic Ca^{2+} of the release of Ca^{2+} from intracellular stores would provide a simple, reliable, and ubiquitous mechanism capable of inducing Ca^{2+} spikes in many different conditions. Although such a mechanism has been demonstrated in muscle and nerve cells as well as in oocytes, its occurrence in other cells in which Ca^{2+} oscillations are found needs to be established. The possibility remains, however, that Ca^{2+} oscillations have not the same origin in all cell types. The present, minimal mechanism for Ca^{2+} oscillations might also operate in conjunction with that proposed by Meyer and Stryer (12) or with a mechanism based on a negative feedback exerted on InsP_3 production (2, 9), which both rely on concomitant oscillations of InsP_3. In either case, such coupling could be the source of the complex Ca^{2+} transients that are sometimes evoked by external stimulation (2, 9, 10).

As to the physiological role of Ca^{2+} oscillations, the progressive increase in the frequency of Ca^{2+} spikes upon increasing the external stimulation could translate into the rising level of some phosphorylated protein that would be the substrate of a Ca^{2+}-activated kinase. We showed that this encoding process is effective only when appropriate conditions hold for the kinetics of the kinase and phosphatase involved in the covalent modification. Encoding signals in terms of the frequency of Ca^{2+} oscillations provides an alternative to the encoding based on varying the amplitude of a steady level of cytosolic Ca^{2+}, whose precise modulation by external signals might prove more difficult to achieve. Moreover, Ca^{2+} oscillations allow for selective differential stimulation of cellular processes (2), since the occurrence of a particular response will depend on the rates of the phosphorylation and dephosphorylation reactions controlled by Ca^{2+} transients.

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